UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/503,758	02/14/2000	William G. Thilly	2909.1000-004	7123

21005 7590 01/08/2004

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.
530 VIRGINIA ROAD
P.O. BOX 9133
CONCORD, MA 01742-9133

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	25

DATE MAILED: 01/08/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/503,758	THILLY, WILLIAM G.	
	Examiner	Art Unit	
	Teresa E Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 April 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-65* is/are pending in the application.

4a) Of the above claim(s) *1-22,24,29-32 and 34-58* is/are withdrawn from consideration.

5)☒ Claim(s) *23 and 61-65* is/are allowed.

6)☒ Claim(s) *25-28, 33, 59 and 60* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This office action is in response to an amendment filed on April 1, 2003. Claims 1-60 were previously pending, with claims 1-22, 24, 29-32 and 34-58 withdrawn from consideration. Applicant amended claims 23, 25, 26, 33 and 59, and added new claims 61-65. Claims 1-65 are pending, with claims 1-22, 24, 29-32 and 34-58 withdrawn from consideration.

2. Applicant's amendments and arguments overcame the rejection of claims 23, 25, 26, 33, 59 and 60 under 35 U.S.C. 112; second paragraph. Applicant's arguments regarding rejection of claims 25, 33 and 59 under 35 U.S.C. 102(b) over Kervinen et al. and rejection of claim 60 under 35 U.S.C. 103(a) over Kervinen et al. and Khrapko et al. have been considered, but are moot in view of new grounds for rejection.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 61 and 63-65 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or

guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention and breadth of claims

Claims 61and 62-65 are broadly drawn to methods of identifying inherited point mutations by identifying their frequencies, obtaining sums of the frequencies and determining whether the gene carries a recessive allele (if the sum of the frequencies of all point mutations is from about 0.02% to 2%) or a dominant allele (if the sum of the frequencies of all point mutations is less than about 0.02%). In case of claim 61, the allele is one which interferes with reproduction, in case of claims 63-65 the allele is a deleterious allele. However, as will be further discussed, there is no support in the specification and prior art for these methods as claimed. The invention is a class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

Working Examples

The specification has no working examples of how to determine the frequency of inherited point mutations in a population. Further, no evidence is provided of even a single gene, which, when all of the frequencies of the point mutations are summed up, turns out to be either dominant or recessive (either deleterious or interfering with reproduction). On page 141 (lines 14-28) and page 142, for example, applicant describes determination of point mutations in short stretches (from 66 to 121 bp) of exon 15 of the APC gene and exons 2-9 of the Hprt gene. One point mutation was found in exon 15 of the APC gene, at a frequency of 11% (Table 7). In the Hprt gene, one point mutation was found in exon 6 at a frequency of 0.006, or 0.6%, and four point mutations with frequencies from 0.2 to 0.8% in exon 9 (Table 2). When these frequencies are added up (assuming 0.8% each for the exon 9 mutations), the sum is 3.8%. Applicant is silent as to the meaning of these number in terms of determining whether such alleles are recessive or dominant. Therefore, there is no support

in the examples cited for the range of frequencies claimed as determining whether the mutation is recessive or dominant.

Guidance in the Specification.

The specification provides no evidence that the claimed methods would indeed determine whether a given gene allele would be recessive or dominant. The guidance provided by the specification amounts to an invitation for the skilled artisan to try and follow the disclosed instructions to make and use the claimed invention. The specification discloses a theoretical model predicting a distribution of mutations in a population at risk for pancreatic cancer (Example 1). On page 37, for example, Applicant cites some experimental data on the mutations in Hprt gene and APC genes. In the Hprt gene, frequencies of individual point mutations were found to range from 0.01% to more than 10 % (lines 5-7), and frequencies of individual SNPs in the APC gene ranged from 1 to 40% (lines 19-21). Applicant calculates expected frequencies of mutations based on the monogenic or polygenic models (pages 38-40). Example 2 presents a theoretical model colon cancer mortality based on assumptions about cancer progression, mutation rates and mutation frequencies in humans, mortality rates, etc. Example 3 provides theoretical estimates of deleterious point mutation frequencies, however, the foundations of these estimates are not clear. For example, on page 132, Applicant states, "Recessive deleterious alleles are expected to be carried by 1.33% of the population" (line 9). It is not clear how this number was obtained. This number is then used to estimate a possible number of alleles, which account for fetal wastage. Further, on page 133, Applicant states "For a proposed random set of 15 genes we might thus expect 3 or fewer to show a set of obligatory knockout alleles with fractions summing to about 0.3% permitting the inference that such a gene carries recessive deleterious alleles." (lines 7-10). It is not clear how this value was obtained. On page 135, we have "Unfortunately, the use of even a thousand blood samples will

not reveal recessive deleterious mutations such as many of those causing cystic fibrosis and a suspected several thousand forms of fetal loss. This is because the sum of fractions of such deleterious alleles is expected to be about 1.33% since individual alleles are expected at fractions ranging from 0.3% to 0.003%." (lines 11-15). Again, the origins of the 1.33% and the expected individual frequencies of 0.3% to 0.003% are not clear.

In summary, the origins of the claimed frequency ranges for the determination of whether the allele is recessive or dominant are not supported by the specification.

The unpredictability of the art and the state of the prior art

The prior art suggests that determination of mutation frequencies and modes of inheritance is not straightforward. Davies et al. (in "Molecular Basis of Inherited Disease", IRL Press, pp. 21-25, 1992), points to the fact that determination of the type and presence of inheritance is not easy. "First, since parents give their children both their genes and their environment, the fact that a character tends to run in a families does not prove that it is genetic-it might, for example, be due to a bad diet. Secondly, most characters depend on interactions between one or more gene loci, conferring susceptibility, and an environmental trigger. Such characters are exceedingly refractory to current methods of genetic analysis; despite their great clinical importance, very little progress has been made in the genetic analysis of schizophrenia, depressive psychosis or other major diseases." (page 20, last paragraph; page 21, first paragraph). Davies et al. then give definitions of dominant and recessive inheritance, pointing to the fact that "...dominance and recessiveness are properties of the observed characters, not of genes. People often talk loosely of dominant or recessive genes, but this is to be discouraged. Sickle cell anemia is a recessive disease (it is seen

only in HbS homozygotes but not in heterozygotes), but sickling trait is dominant, because it is apparent in the heterozygote." (page 21, third paragraph; emphasis added).

Davies et al. further define what diseases are considered as autosomal dominant (page 21, last paragraph; page 22, Fig. 2.5, 2.6), autosomal recessive (page 23; Fig. 2.7), X-linked dominant and recessive (page 24, 25; Fig. 2.8, 2.9). For example, in most rare diseases homozygotes have never been described because the necessary marriage of two affected heterozygous individuals has not occurred, or because they die in utero (page 22, last paragraph, continued on page 23). Davies et al. also cautions that interpretation of pedigrees is complicated by factors such as variable expression (a condition can be severe in one person and mild in another) or non-penetrance (the person has no sign of a condition despite carrying a gene inherited from a parent) (page 22, second paragraph; Fig. 2.6). Therefore, according to Davies et al., reference to gene as recessive or dominant is not a proper use of established terminology, and classifying conditions as dominant or recessive is not clear-cut.

Crow et al. (Adv. Human Genetics, vol. 14, pp. 59-123, 1985) also describe a mutation as being dominant if the mutant phenotype that is observed is that of a heterozygote (page 61, last paragraph). However, as they point out, the distinction between "recessive" and "dominant" is not a clear one: "There is good evidence in experimental animals that most "recessive" mutations are actually partially dominant (i.e., have some effect on the heterozygote), and the human evidence, as far as it goes, is consistent with this observation" (page 62, second paragraph). Crow et al. also point to the fact that even though it is quite clear that mutations that produce overt effects are almost always harmful, it is not clear whether mutations which result in no detectable phenotypic changes may be classified as harmful or not (page 61, second paragraph). They echo Davies et al. in the

view that classification of diseases according to pedigrees is not clear-cut, leading to underrepresentation of disorders classified as recessive (page 64, second and third paragraphs).

Crow et al. teach that determination of dominant phenotypes is prone to errors, such as determination of incidence dominant phenotypes in children, errors in paternity determination, incomplete penetrance and a presence of a recessive form of the same phenotype. These errors lead to an overestimate of the mutation rates (page 66, paragraphs 2-4; page 67, paragraphs 1-4). On the other hand, the number of individuals carrying such mutations is underestimated because of deaths of a large number of affected individuals (page 67, fifth paragraph). In the case of dominant phenotypes, indirect methods of measuring the mutation rates can be applied, but, as pointed out by Crow et al., both direct and indirect methods should be used for greater confidence (page 75, last paragraph). As for the recessive mutations, Crow et al. teach that the frequencies of such mutations are influenced by several factors, including numbers of cosanguineous matings, heterozygote selection and levels of inbreeding (page 76, third paragraph).

Conneally (Dev. Biol. Stand., vol. 83, pp. 107-110, 1994) teaches that measurement of mutation rates in a population is difficult because of the rarity of mutations, with the mutation rates mutations linked to X-linked recessive diseases being the easiest to estimate. However, even in such cases errors lead to unreliable estimates (page 110, third paragraph).

Cavalli-Sforza et al. (in The Genetics of Human Populations, W.H. Freeman and Company, San Francisco, 1971, pp. 71-110), discuss deleterious mutations. They teach that such mutations are subject to selection, which eliminates the mutant genes when their frequency increases (page 72, paragraphs 3 and 4). They estimate equilibrium frequencies of recessive deleterious and dominant deleterious mutations assuming equilibrium between arising new mutations and selection. For a deleterious recessive gene, the gene frequency is estimated as $\sqrt{\mu/s}$, where μ is the mutation rate and

s is the selection coefficient (page 79, last paragraph; page 80; page 81, first and second paragraphs). For a lethal gene, s = 1, and if the mutation rate is of the order of 10^{-6}, the frequency of the gene is 0.1%, whereas with a very high mutation rate of 10^{-4}, the gene frequency would reach 1%.

Finally, examples of particular diseases and mutation rates, and difficulties associated with a reliable estimate of gene frequencies. De la Chapelle (J. Med. Genet., vol. 30, pp. 857-865, 1993) describes mapping of genes associated with certain diseases in isolated human populations in Finland. In particular, they teach that rare dominantly inherited diseases can be highly enriched in isolated populations if they do not confer selective disadvantage (page 859, third paragraph). The same is true for autosomal recessive mutations (page 861, 6th full paragraph). Therefore, mutation frequencies in isolated populations might be much higher than in general population for the same type of mutation (dominant or recessive). Cooper et al. (Hum. Genetics, vol. 85, pp. 55-74, 1990) cite a frequency of an autosomal dominant disorder, von Willebrand's disease, as being 0.82% in the general population (page 67, third paragraph). Further, the same condition can be transmitted in a different manner. Hardelin et al. (Hum. Mol. Genetics, vol. 2, pp. 373-377, 1993) describe that Kallman syndrome can be transmitted as X-chromosome linked, autosomal dominant and autosomal recessive (page 373, second paragraph).

In summary, the prior art does not support the assumption that sum of frequencies of point mutations of the order of 0.02% to 2% indicates recessive alleles and sum of frequencies of point mutations of less than 0.02% indicates dominant alleles.

Quantity of Experimentation

The quantity of experimentation in this area is extremely large since there is significant number of parameters, which would have to be studied to apply this method to determination of recessive and dominant alleles, which are harmful or interfere with reproduction. At least one study would need to be performed in the general population by sequencing at least one gene, determining all of the point mutations in such gene, determining which one of them are obligatory knockout point mutations (i.e., mutations which render the gene inactive, according to Applicant's definition on page 25, lines 10-13). Gene inactivation may mean that the gene is not expressed at all (due to the mutation in a promoter region, for example), or has residual or no activity. In the latter case activity of the proteins expressed from the mutated DNA would have to be determined to find out whether the point mutations are indeed obligatory knock-out mutations. Since these mutations should be inherited, additional effort would be required to genotype all of the individuals' parents and/or siblings, to determine which one of such point mutations are inherited. Further, since dominant deleterious mutations usually result in removal of the affected individuals from populations, they may not be detectable at al. Therefore, to detect very rare mutations, extremely large populations would need to be screened. This would require years of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

Conclusion

In the instant case, as discussed above, in a highly unpredictable art where the detection of mutation frequencies depend upon numerous known and unknown parameters such as the population size, selection, mutation rate, etc., the factor of unpredictability weighs heavily in favor of undue experimentation. Further, the prior art and the specification provides insufficient guidance

to overcome the art recognized problems in the use of mutation frequencies to determine whether a given harmful allele is recessive or dominant. Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the absence of a working example and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 23, 25-28, 33 and 59-65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

7. Claims 23, 25-28, 33 and 59-65 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: a step of determining whether the point mutations are inherited. Applicants did not define what it means for a point mutation to be inherited, or how to determine whether it is. For example, is a mutation which occurs in an egg (before or after fertilization), but is not present in mother's germline, inherited? Is the term confined to Mendelian inheritance or does it encompass non-Mendelian inheritance as well?

B) Claims 23 and 25 are indefinite because it is not clear whether the method identifies one gene which carries one or more harmful allele, or multiple genes, each of which carries one or more

harmful allele. In steps a) and b), mutations are determined for one or more genes, whereas in step c) a comparison of the sum of frequencies of point mutations is made for a selected gene.

C) Claims 26-28 are indefinite in claim 26, because claim 26 is indefinite over the recitation of "the age-specific decrease of said two or more point mutations" in line 1 of step e). It is not clear what it means for a mutation to decrease (does it mean decrease in the number of mutations?).

D) Claims 26-28 are indefinite in claim 26, because claim 26 lacks antecedent basis for the following limitations:

a) "the age-specific decrease in frequency" in line 1 of step f) and in line 10; step e) has "the age-specific decrease of said two or more point mutations";

b) "the functions" in line 3 of step f);

c) "said one or mortal diseases" in line 15.

E) Claim 61 is indefinite because the preamble does not seem to correspond to the final method step. In particular, the preamble states "A method of identifying one or more inherited point mutations", whereas in step a) we have "determining the set of all inherited point mutations", which would imply at least two point mutations. In step c), the sums of frequencies of point mutations are calculated, again implying determination of more than one point mutation.

F) Claim 61 is indefinite because it is not clear how the sums of the obligatory knock-out point mutations are calculated. In step b), frequencies of point mutations are calculated within and between the subpopulations. It is not clear whether the sums of the frequencies are calculated for each subpopulation or whether they are calculated for the whole population.

Claim interpretation

8. A) Applicant did not define the term "inherited point mutation", therefore it is interpreted as any point mutation.

9. B) In claims 23, 59 and 62, since the minimum point mutation number is one and a minimum number of genes is also one, the sum of the frequencies is identical to a frequency of just one mutation in one gene.

C) The term "obligatory knock-out point mutation" has been defined by Applicant as point mutations which necessarily inactivate the gene (page 25, lines 10, 11). However, Applicant did not define what it means for a gene to be inactive. Therefore, any point mutations within a gene are considered as obligatory knock-out mutations, since potentially they can change protein activity.

Claim Rejections - 35 USC § 102

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

11. Claims 23 and 62 are rejected under 35 U.S.C. 102(b) as being anticipated by Kervinen et al. (Atherosclerosis, vol. 105, pp. 89-95, 1994; cite in the IDS; cited in the previous office action), as evidenced by Margaglione et al. (Stroke, vol. 29, pp. 399-403, February 1998).

Regarding claims 23 and 62, Kervinen et al. teach identification of a harmful alleles, the method comprising:

a) identifying one or more inherited point mutations that are found in one or more genes or portions thereof of a population of young individuals, determining the frequency with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment (Kervinen et al. teach identifying frequencies of apolipoprotein E (apo E) and apolipoprotein B (apo B) polymorphisms in populations of young adults (Abstract; Fig. 2; Table 3, 4).);

b) identifying one or more inherited point mutations that are found in one or more genes or portions thereof of a population of aged individuals, determining the frequency with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment (Kervinen et al. teach identifying frequencies of apolipoprotein E (apo E) and apolipoprotein B (apo B) polymorphisms in populations of nonagenarians (Abstract; Fig. 2; Table 3, 4).);

c) comparing the sum of the frequencies of point mutations that are found in a selected gene or portion thereof of the young population calculated in a) with the sum of the frequencies of point mutation that are found in the same gene or portion thereof of the aged population calculated in b), wherein a significant decrease in the sum of the frequencies of point mutations in the aged population indicates that said selected gene carries one or more harmful allele (Kervinen et al. teach comparing the frequencies of apoE ε4 allele and the EcoRI R- apoB allele between the young adults and nonagenarians, and finding that frequencies of these two alleles were significantly lower in the nonagenarins than in young adults (Fig. 2, Table 4; page 92, last paragraph; page 93). Kervinen et al. conclude that the presence of apoE ε4 allele is a major risk factor for coronary heart disease (CHD) (page 93, last paragraph), and suggest that the R- allele may be a risk factor for a CHD (page 94, fourth paragraph).

Regarding claim 62, Kervinen et al. do not specifically teach that apoE polymorphisms are knock-out point mutations (or point mutations which inactivate the gene, according to Applicant's definition). Margaglione et al. teach that that the apoE ε4 allele results in the amino acid substitution of Cys -> Arg at position 112. Therefore Kervinen et al. anticipate this limitation of claim 62.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 25, 33, 59 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kervinen et al. (Atherosclerosis, vol. 105, pp. 89-95, 1994; cite in the IDS; cited in the previous office action), Khrapko et al. (1) (Nucl. Acids Res. Vol. 25, pp. 685-693, 1997; cited in the previous office action) and Khrapko et al. (2) (Nucl. Acids Res. Vol. 22, pp. 364-369, 1994; cited in the previous office action), as evidenced by Margaglione et al. (Stroke, vol. 29, pp. 399-403, February 1998).

A) Regarding claim 25, 33 and 59, Kervinen et al. teach identification of a harmful allele of apolipoprotein E (apo E) and apolipoprotein B (apo B) by:

a) identifying the set of inherited point mutations that are found in one or more genes or portions thereof of a population of young individuals, wherein the set comprises all inherited point mutations occurring at a frequency at about or above 5x10-5, and determining the frequency with which each point mutation occurs (Kervinen et al. teach identifying frequencies of apolipoprotein E (apo E) and apolipoprotein B (apo B) polymorphisms in populations of young adults (Abstract; Fig. 2; Table 3, 4).);

b) identifying the set of inherited point mutations that are found in the genes genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs (Kervinen et al. teach identifying frequencies of apolipoprotein E (apo E) and

apolipoprotein B (apo B) polymorphisms in populations of nonagenarians (Abstract; Fig. 2; Table 3, 4).);

c) comparing frequency of point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of two or more point mutations in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele (Kervinen et al. teach comparing the frequencies of apoE ε4 allele and the EcoRI R- apoB allele between the young adults and nonagenarians, and finding that frequencies of these two alleles were significantly lower in the nonagenarins than in young adults (Fig. 2, Table 4; page 92, last paragraph; page 93). Kervinen et al. conclude that the presence of apoE ε4 allele is a major risk factor for coronary heart disease (CHD) (page 93, last paragraph), and suggest that the R- allele may be a risk factor for a CHD (page 94, fourth paragraph)).

B) Kervinen et al. do not teach detection of mutations occurring at a frequency at about or above 5 x 10-5 or constant denaturant capillary electrophoresis (CDCE) combined with high-fidelity PCR to determine point mutations in DNA samples from populations.

C) Khrapko et al. (1) teach a method of determining point mutations occurring at a frequency of 10^{-6} or above in a DNA sample using constant denaturant capillary electrophoresis (CDCE) combined with high-fidelity PCR. The method comprises the following steps:

a) restriction digest of DNA isolated from cells to obtain a 200 bp DNA fragment with low temperature and high temperature isomelting domains,

b) enrichment of mutant sequences by constant denaturant gel electrophoresis (CDGE),

c) high fidelity PCR amplification resulting in fluorescently labeled products, using *Pfu* polymerase, which has an error rate of $2x10^{-6}$ errors per base per doubling,

d) separation of PCR heteroduplexes from homoduplexes by CDCE and collection of the heteroduplxes,

e) another round of high fidelity PCR in which mutant heteroduplexes are converted into homoduplexes by stopping the PCR reaction when the molar amount of unused primers still exceeds the molar amount of the products,

f) another round of CDCE separation of the homoduplexes,

g) isolation and sequencing of the mutants. (Fig. 1; page 686-689).

Khrapko et al. (2) teach that prior to CDCE separation the DNA fragments are boiled and reannealed, resulting in a mixture of homoduplexes and heteroduplexes, which are then separated based on the differences in their melting temperature in a CDCE capillary column (page 365, paragraphs 6-9; page 366; Fig. 3, 4).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the point mutation detection method of Khrapko et al. (1) and (2) in the method of mutation detection of Kervinen et al. The motivation to do so, provided by Khrapko et al., would have been that combining CDCE with high fidelity PCR permitted detection of low frequency mutations (page 685, fifth paragraph).

14. No references were found teaching or suggesting claims 26-28, 61 and 63-65, but they are rejected for reasons given above.

Conclusion

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E Strzelecka whose telephone number is (703) 306-5877. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (703) 308-1119. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

JEFFREY FREDMAN
PRIMARY EXAMINER

TS
January 5, 2004